natally (*1*). We also know that, contrary to previous belief, liver cancers are slow-growing malignancies. The earlier perception that liver cancers are aggressive tumors was simply a result of our limited ability to diagnose them early. In fact, it may take an average of 3 years (range 0.8 to 11 years) for a liver tumor that is 1 centimeter in diameter to grow to a size large enough to cause symptoms (*11*). When symptoms appear, the HBsAg carrier is already at the terminal stage and will usually die within months. In other HBsAg carriers, liver function may be severely compromised by the refractory hepatitis and the patient may die of hepatic failure or other complications of cirrhosis. A study in Taiwan has estimated that as many as 50% of all HBsAg-positive men will die of cirrhosis or hepatoma (*7*).

Our improved understanding of the natural history of chronic type B hepatitis has led to more effective approaches toward the control of this important viral infection and its sequelae (see figure). Most important, immunization against HBV in the perinatal setting has been shown to prevent chronic infections with an efficacy of about 85%. Forty-seven countries currently have national immunization policies that include hepatitis B vaccination of infants (*12*). One of the earliest programs, adopted in Taiwan in 1984 and involving universal vaccination of infants and children, has proved very effective. The percentage of children carrying HBsAg has dropped dramatically from >10 to <2% (*13*). In addition, the data from mothers and vaccinated infants have been stored and will be used to assess whether prevention of chronic HBV infection also reduces the incidence of liver cancer. If it does, then this would be the first time in history that a human cancer has been prevented by mass vaccination.

Although much has been learned from hepatitis B, many important issues remain unresolved. For existing HBsAg carriers, interferons offer some therapeutic benefits but other effective and inexpensive treatments are also needed. The immunologic mechanisms underlying persistent HBV infection, the immunopathogenesis of chronic hepatitis B, and the progression of chronic HBV infections to hepatomas are other issues that remain to be explored in further detail. Molecular biology will continue to play a key role in solving these fundamental questions. In the meantime, efforts to prevent HBV infection—especially by immunization—should continue, with the goal of controlling hepatoma and cirrhosis by the turn of the century.

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High-Pressure Mineral Physics: An Inside View of the Earth

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Our knowledge of the structure of the Earth's interior has been obtained by analyzing seismic waves that travel deep in the Earth. The "solid" Earth (6370-km radius) can be divided into several concentric layers according to the depth profiles of the seismic wave velocities. The primary division is between the mantle of rock and the metallic molten core at a depth of 2890 km. The mantle may be further subdivided into the upper mantle, the transition zone, and the lower mantle by two prominent increases in the velocities found around 400- and 660-km depths. At the center, there is a small inner core (1220-km radius) of solid metal.

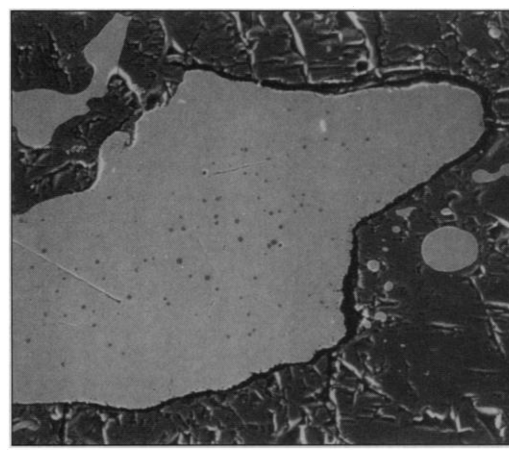

Fig. 1. Secondary electron image of part of a sample quenched at 24 GPa and 2550°C (*19*). The starting material was a mixture of pure iron and $(Mg_{0.9}Fe_{0.1})_2SiO_4$ olivine.

Seismic tomography (*1*) has revealed three-dimensional variations in velocities over most of the mantle and shows descending slabs and the existence of large-scale rising plumes. The goal of high-pressure mineral physics includes interpretation of this seismic information in terms of material science and clarification of the state of the Earth's interior. In the following, I briefly review recent advances by Japanese researchers, but a wide range of high-pressure research is also being carried out at several institutes of the Chinese Academy of Sciences, China, employing both multianvil press and diamond anvil cells (DAC) (*2*). At the Institute of Earth Sciences, Taiwan, DAC experiments are in progress for a variety of topics (*3*). Collaborations under consideration between these and other Asian countries will stimulate new development of high-pressure mineral physics.

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Kimberlite xenoliths, the deepest rocks accessible from the Earth's surface, indicate that the uppermost part of the mantle (down to 150-km depth) is peridotite, composed of mainly olivine associated with Ca-poor and Ca-rich pyroxenes and garnet. Accurate data on the phase equilibria of these mantle minerals at high pressures and temperatures are indispensable to modeling of the mineralogy and chemistry of the deep mantle. Following Akimoto's pioneering work on the olivine-spinel transformation (*4*), Japanese scientists have developed a double-staged, large-volume, multianvil apparatus (*5*). Consequently, phase diagrams relevant to the depth of 800 km in the mantle were constructed in sufficient detail. The phase boundaries have been cross-checked by thermodynamic calculations based on recent measurements of heat of formation and heat capacity for the high-pressure phases (*6*).

Important mineralogical aspects of the

peridotitic mantle are as follows (*5*). The 400-km discontinuity reflects the transformation of olivine into the modified spinel, which successively transforms into the spinel at the depth around 530 km. Pyroxenes dissolve gradually into the garnet to form majorite garnet over the depth range of 300 to 500 km. Therefore, the resultant phases are the major constituents of the transition zone. The 660-km discontinuity is chiefly caused by the dissociation of spinel into an assemblage of (Mg,Fe)SiO_3 perovskite and (Mg,Fe)O magnesiowüstite. Majorite also dissociates into (Mg,Fe)SiO_3 perovskite, $CaSiO_3$ perovskite, and small amounts of an Al_2O_3-rich phase and SiO_2 stishovite. Thus, the magnesian silicate perovskite is by far the most dominant phase in the lower mantle, followed by the relatively iron-rich magnesiowüstite. The temperature at the base of the transition zone is estimated to be about 1600°C from the sharp dissociation boundary of the spinel (*8*).

The peridotitic mantle mineralogy is generally consistent with the seismic mantle models. However, some lines of evidence show that the lower mantle density agrees with that of (Mg,Fe)SiO_3 perovskite alone rather than that of perovskite plus magnesiowüstite with the peridotitic proportion (9). Therefore, the chemical composition of the lower mantle is still in debate. In any case, more than 75% of the lower mantle is likely to be silicate perovskite and therefore this material is the most dominant constituent of our planet.

Recently, Irifune and Ringwood (*10*) showed that the preservation of majorite in the subducted mid-oceanic basalt to the lower mantle would cause the stagnation of descending slabs along the 660-km discontinuity (*1*). The role of phase transformations in mantle convection and plumes is a subject for investigation in the next decade. As carried out by Kamaya *et al.* (*11*), the application of high-pressure phase equilibria to the interior of other terrestrial planets will also be an important contribution to the comparative planetology.

The diamond cell is not only the method of choice for creating pressures higher than 100 GPa, but it also makes spectroscopic measurement and x-ray diffraction feasible. Numerous high-pressure transformations were discovered by means of a DAC coupled with laser heating (*12*). Tsuchida and Yagi (*13*) observed the reversible transformation of SiO_2 stishovite to the $CaCl_2$ structure at 108 GPa by laser heating and subsequent x-ray probing of the sample in DAC under high pressure. The polymorph that was found should be stable deep in the lower mantle.

Since 1980, a multianvil press (MAX80) combined with intense x-ray synchrotron radiation at the National Laboratory for High Energy Physics (KEK) in Tsukuba (*14*) has been extensively used to determine physical properties and phase boundaries under the precisely controlled pressures. Recently, the capability of MAX80 was considerably extended when the tungsten carbide anvils were replaced with sintered diamond. On the basis of this technical development, Funamori and Yagi (*15*) demonstrated that the orthorhombic symmetry of $MgSiO_3$ perovskite is preserved in the lower mantle, contrary to the predicted transformation of this phase to higher symmetry. They also determined the thermal expansivity of $MgSiO_3$ perovskite under conditions within its stability field.

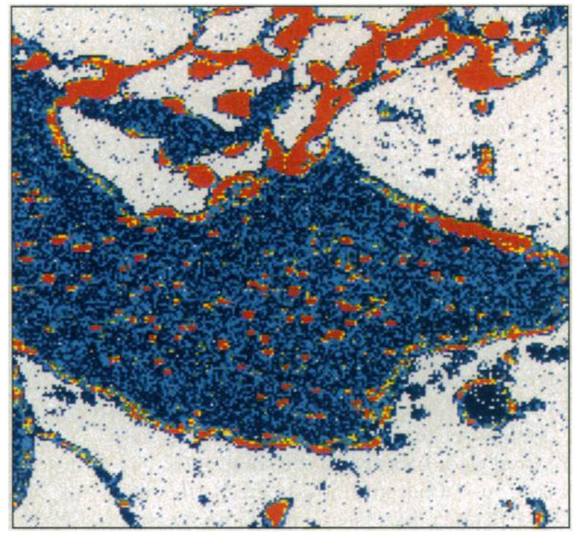

Fig. 2. Elemental mapping (Si) of the sample shown in Fig. 1. Blue, red, and white portions are "molten" iron, silicate melt, and magnesiowüstite. The molten iron contains about 2% Si.

The phase equilibria and equations of state for the mantle minerals determined by in situ observation are indispensable to a better understanding of the dynamic nature of Earth's deep interior as well. In situ x-ray diffraction provides insight into the mechanism of high-pressure transformation, which is important for elucidation of the origin of deep-focused earthquakes. A new photon factory of 8 GeV (Spring-8) under construction at Harima, Japan, will further advance this kind of research.

The evolution of the early Earth has become a key topic in high-pressure mineral physics. It has been generally accepted that the proto-Earth had been greatly heated by the liberated gravitational energy on accretion, and hence, it was covered by a completely or partially molten layer extending to more than 1000-km depth (the magma ocean). A large-scale differentiation caused by fractional crystallization would have proceeded during formation or solidification of the magma ocean, resulting in a chemical layering of the mantle (*16*). A notable difference in Mg/Si ratio between the upper mantle periodotite (~1.4) and Cl chondrite (~1.0), which is regarded as similar to primary source material of the Earth, might be reconciled by the fractionation of majorite or perovskite to the lower mantle, leaving an ultrabasic liquid as the upper mantle.

Melting studies on mantle peridotite (*17*) and model mantle material derived from the C1 chondrite composition (*18*) to 25 GPa have revealed that majorite and perovskite appear as the liquidus phases at pressures higher than 15 GPa, supporting the fractionation hypothesis in the magma ocean. Nevertheless, the partitioning data of major and trace elements among melt phases indicate that the upper mantle peridotite shows no evidence of the gross differentiation that is seen in perovskite and majorite (with respect to the relative abundance of elements compared with that of C1 chondrite) (*19*). The possibility of stratification of the primitive mantle should be investigated in more detail especially with regard to the following. The liquidus phase might be strongly composition-dependent and magnesiowüstite as the liquidus phase could be possible. The possible density crossover between liquid and solid at high pressure (*20*) is crucial for the differentiation and should be clarified in detail.

It is generally accepted that most of the core was formed in a quite early stage of the Earth's history either during the accretion or very soon afterward during about 100 to 500 million years. The core formation is undoubtedly the most important event in the Earth's history because it separated the Earth into two almost closed systems, the mantle and the core. In other words, the core formation set the initial conditions for the later evolution of the Earth.

The outer core is believed to be composed of molten iron alloying with substantial amounts of lighter elements because its density is about 10% less than that of pure iron under corresponding pressure and temperature conditions. Several candidate elements, such as Si, S, O, C, or H, have been proposed on the basis of cosmic abundance or solubilities in iron at high pressure and temperature.

According to the magma ocean hypothesis, core segregation had rapidly proceeded as molten iron sank through the completely or partially molten silicates. Ito and Katsura (*21*) showed that Si, which may form up to 2% of the core, together with O, dissolves into molten iron from silicate melt at 24

GPa and 2550°C (Figs. 1 and 2) and that the dissolution of Si is enhanced with increasing pressure. Therefore, Si and O could be important light elements in the core. Concurrently, the SiO_2 content of the mantle would be substantially reduced from its primitive one by core formation in the deep magma ocean. In the experiments, however, entry of Mg into molten iron was not observed. Thus, the core formation should have strongly affected the chemistry of the mantle. The light elements that were incorporated, on the other hand, certainly play an important role in the dynamics and evolution of the core.

High-pressure mineral physics has extensively contributed to the solution of the question, "What and how is the Earth's interior?" The answer generates more specific and comprehensive new questions, "Why and when was the answered situation caused?" This is because the Earth has evolved for 4.5 billion years, and its present status reflects all the time-integrated results of various events and complicated processes.

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Proteases in *Escherichia coli*

Chin Ha Chung

Cells make mistakes. Sometimes the proteins of bacterial and animal cells are not synthesized correctly. This can happen by accident through biosynthetic error or mutation, or can be induced by the incorporation of amino acid analogs or by stresses such as high temperatures (*1*). Because such abnormal polypeptides with highly aberrant structures are nonfunctional and are frequently toxic to cells, it is to the cell's advantage to eliminate them. This essential cellular process is accomplished by the action of proteolytic enzymes. The mechanisms by which these enzymes digest abnormal proteins have been illuminated by the study of the proteases of the bacterium *Escherichia coli*.

Since the early 1980s, nine distinct endoproteases have been isolated from *E. coli* (see table) (*2*). Seven of these, proteases Do, Re, Mi, Fa, So, La, and Ti, are serine proteases that hydrolyze large proteins such as casein and globin. Two other enzymes, proteases Ci and Pi, are metalloproteases that degrade smaller polypeptides, such as insulin and short amino-terminal fragments of β-galactosidase. Proteases Mi and Pi are periplasmic enzymes, while all others are cytoplasmic and therefore may potentially degrade intracellular proteins.

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Protease Do is a serine protease with an unusually high molecular mass of about 500 kD (*3*). This enzyme in vitro catalyzes limited cleavage of the Ada protein, which takes part in the repair of methylated DNA, and of the IciA protein, which is an inhibitor of replication initiation of the *E. coli* chromosome (*4*). In addition, protease Do is identical to the *htrA* gene product, and mutations of the gene result in the loss of cell viability at high temperatures and loss of its ability to degrade alkaline phosphatase fusion proteins (*5*). Proteases Re and So degrade oxidized glutamine synthetase but not the native form of the protein (*6*). Protease So also degrades signal peptides after their release from precursor proteins in vitro (*7*). In addition, protease Re has recently been identified as Tsp (tail-specific protease), which in vitro catalyzes the degradation of a bacteriophage λ repressor variant (*8*).

An intriguing feature of protein breakdown in bacteria, as well as in animal cells, is its requirement for metabolic energy (*1*). For example, in *E. coli*, inhibitors of energy metabolism greatly reduce hydrolysis of most abnormal proteins and certain regulatory proteins. This energy requirement would not be expected on the basis of thermodynamics or from the properties of typical proteolytic enzymes. Studies on the mechanistic basis of this energy requirement have led to the isolation of two *E. coli* proteases, La and Ti, which are dependent on adenosine triphosphate (ATP) and Mg^{2+} for activity.

The protease La is the product of the *lon* gene (*9*). This enzyme is essential for the degradation of most abnormal proteins and certain normal short-lived polypeptides, in-

Soluble endoproteases from *E. coli*. Abbreviations: DFP, diisopropyl fluorophosphate; NEM, *N*-ethylmaleimide; and GS, glutamine synthetase.

Protease	Gene	Size (native) (kD)	Substrate	Inhibitor	ATP-dependency
Do	*htrA*	48 (500)	Casein, IciA, Ada	DFP	No
Re	*tsp*	82 (82)	Casein, oxidized GS	DFP	No
Mi		110 (110)	Casein	DFP	No
Fa		110 (110)	Casein	DFP	No
So		77 (140)	Casein, oxidized GS, signal peptides, Ada	DFP	No
La	*lon*	87 (450)	Casein, SulA, RcsA, λN	DFP, NEM, ADP	Yes
Ti	*clpA*	84 (140)		NEM	Yes
	clpP	21 (240)	Casein, ClpA	DFP	
Ci		54 (54)	Insulin	*o*-phenanthroline	No
Pi	*ptr*	110 (110)	Insulin	*o*-phenanthroline	No